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Award Number:
W81XWH-08-2-0082

TITLE:
A Blast Model of Traumatic Brain Injury in Swine

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REPORT DATE:
November 2009

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

X Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*
1 November 2009

2. REPORT TYPE
Annual

3. DATES COVERED *(From - To)*
15 APR 2008 - 14 OCT 2009

4. TITLE AND SUBTITLE
A Blast Model of Traumatic Brain Injury in Swine

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-08-2-0082

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Northern California Institute for Research and Education

San Francisco, CA 94121

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command

Fort Detrick, MD 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The purpose of this study is to develop a survival model of blast-induced traumatic brain injury (BI-TBI) in swine. Two air guns have been constructed, each having different lengths, air chamber volumes and barrel diameters. Using a digital ballistics chronometer the air velocity was measured over a series of firings at different controlled pressures in the air chambers. Calibration curves from both air guns show linear and reproducible results through a range of firing pressures. Behavioral/learning/memory testing has been developed which will detect cognitive and behavioral changes for up to 2 months following BI-TBI. To date swine have received BI-TBI with the air guns of two different sizes and were recovered for 7 days post injury. BI-TBI with the larger gun caused initial pain requiring analgesia and reduced weight gain compared to injury generated with the smaller gun. Brains will be examined for molecular markers of injury. Histology and immunocytochemistry will evaluate cytoarchitecture, degenerating neurons and morphology. When completed this swine model BI-TBI will provide a needed tool for use in developing treatment and rehabilitation of traumatic head injuries as well as improvements in military protective body armor.

15. SUBJECT TERMS
Traumatic Brain Injury, Swine, Blast, Model Development

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	8	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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Annual Report for project W81XWH-08-2-0082
A blast model of traumatic brain injury in swine.

INTRODUCTION:

Although blast-induced traumatic brain injury (BI-TBI) is a significant cause of morbidity and behavioral dysfunction in war fighters returning from Iraq, laboratory models are not currently available to study the mechanisms underlying this critical injury and develop new therapies to treat survivors. Many TBI models are performed in rodents, and data from these models have been used as a basis for several disappointing Phase III clinical trials in humans with TBI. The failure of these trials may, in part, be due to differences between the rodent and human (or pig) brain. The development of a large-animal model of BI-TBI will revolutionize the study of this pressing clinical problem and rapidly facilitate the development of novel therapies to treat injured military personnel. Therefore, the purpose of these experiments is to develop a survival model of BI-TBI in swine.

BODY:

Protocol Approval:

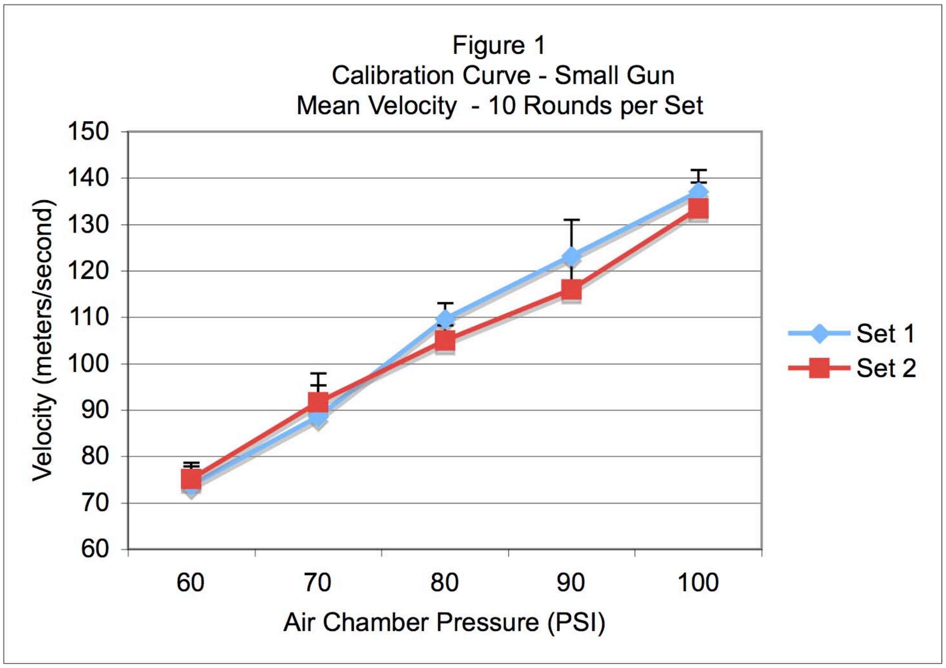
The protocol was submitted to the local IACUC and approval received on 20 March 2008. The protocol was then submitted to DOD on March 2008. The protocol PT 08-18-01 (ACORP) was approved by the ACURO on 7 July 2009.

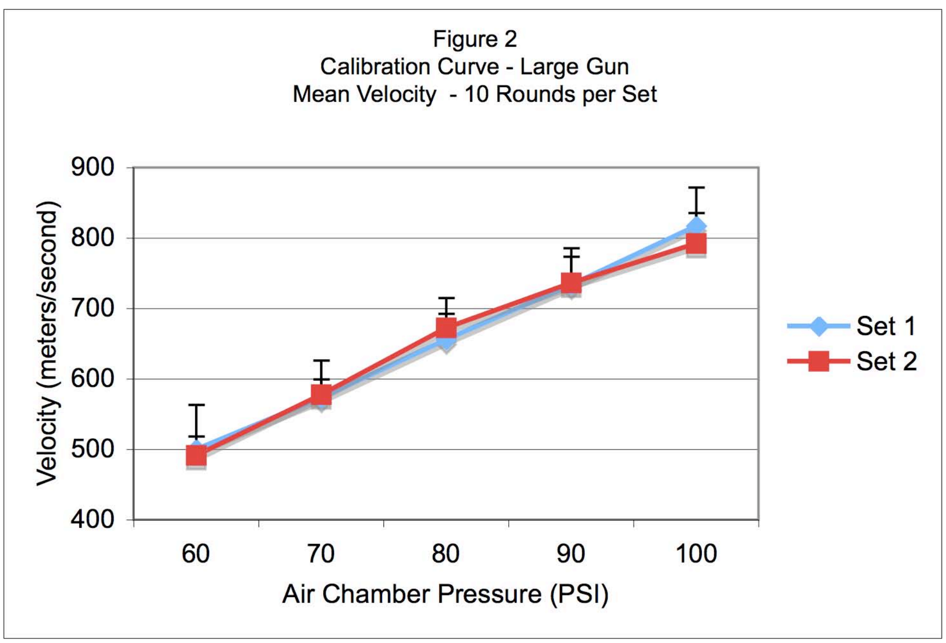
Calibration of air guns:

Two air guns were constructed from PVC piping, each having different lengths, air chamber volumes, barrel diameters and both designed to hold up to 100 pounds per square inch (psi) of pressurized air. The smaller gun has a 40-inch barrel with an internal diameter of one inch. The compression chamber is 26 inches in length and has an outer diameter of 4 inches. The larger gun has a barrel 54.25 inches in length with an inside diameter of 2.5 inches. The compression chamber is 4 inches in outside diameter with a length of 36 inches.

Using a digital ballistics chronometer, which measures the speed of an object such as a bullet, pellet, or an arrow through its sensing field, and small pellets the air velocity was measured over a series of controlled pressures in the air chambers. To characterize the guns and determine the velocity range, each gun underwent two sets of 10 round firings at 60, 70, 80, 90, and 100 psi (using room air and a compressor) in the air chamber. Air velocity in meters/second was measured at a distance of one foot from the end of the gun barrel. The test results of the first set of 10 rounds show the small and large guns produced a mean velocity in meters/sec of 73.9 (± 4.67) and 499.8 (± 63.02), respectively, using 60 psi; 88.6 (± 6.69) and 573.2 (±26.14) at 70 psi: 109.7 (± 3.37) and 656.3 (± 36.24) at 80 psi: 123.2 (± 7.76) and 733.7 (± 39.45) at 90 psi and 137.1 (± 4.63) and 817.2 (±54.37) at 100 psi. A second set of 10 rounds were fired. The test results for the second set of rounds show the small and large gun produced a mean velocity of 75.1 (± 2.78) and 491.1 (± 26.67); under 60 psi respectively; 91.7 (± 6.23) and 577.5 (± 48.10) at 70 psi; 105.0 (± 3.29) and 672.1 (± 42.47) at 80 psi ; 116. (± 6.99) and 736.1 (± 49.32) at 90 psi and 133.4 (± 5.63) and 792 (± 43.46) at 100 psi. Figure 1 shows the mean

velocity in meters/second for both sets of rounds for the small gun and Figure 2 shows the results for the large gun. These calibration curves show linear and reproducible results that quantify known air velocities through the use of air blast guns.





Behavioral/learning/memory evaluation:

During a previous study with swine we have utilized a learning/memory paradigm where the animals were given 5 minutes in a room with three identical boxes (of different colors: white, yellow, and blue) containing food. Only the blue box could be opened to retrieve food. The animals were expected to learn (and remember) to go directly to the blue box and open it to obtain food, without attempting to open other boxes. We monitored the number of sessions taken to learn the task, time taken to finish the task (max. 300 s) during each session, and a composite performance score that was calculated as follows:

+2 = opens blue box
+1 = touches blue box but does not open it
+1 = does not approach the other two boxes
+0 = smell either of the other two boxes
-1 = tries to open either of the other two boxes

The maximum score possible was +3 (opening of blue box without approaching the other two boxes). Each false attempt earned a negative mark (no maximum). The session ended as soon as blue box was opened or at 300 seconds. We are now confident that we can detect changes in learning/memory caused by BI-TBI in this study by applying this paradigm. We also monitored neurological status daily utilizing a neuroscore based on a 500-point scale (0 = normal; 500 = brain dead). We have gained valuable experience to apply to this study.

Blast Induced Traumatic Brain Injury:

Procedure: Four female Yorkshire swine have been used thus far, two were injured with the smaller gun at 100 psi, one was injured with the larger gun at 80 psi and one sham (no injury). The animals were premedicated with ketamine i.m. (20 mg/kg) and glycopyrrolate i.m. (0.1 mg/kg) then masked with 4% isoflurane to achieve a deep plane of anesthesia, at which time they were intubated with an endotracheal (ET) tube. Anesthesia was maintained with isoflurane (1-4%) using standard anesthesiology apparatus to deliver inhalation anesthetics. Once deeply anesthetized, the animals were placed in lateral recumbency and immobilized firmly on a table with 2-inch-wide nylon straps with Velcro fasteners. The heads of the animals were capable of movement. The animals were monitored for blood oxygen saturation. Protective goggles were placed over the swine's eyes. The air gun was secured to a nearby table and positioned with the barrel perpendicular to and centered between the ears and eyes of the swine with the barrel opening 5 cm from the top of the skull. Immediately prior to firing of the gun, the ET tube was disconnected from the anesthesia apparatus. The gun was fired, then the anesthesia tubing reconnected. This “blast” caused an acceleration/deceleration injury to the swine brain. When the animal was stable the anesthesia was gradually reduced and the animal permitted to recover from anesthesia. She was moved to a mobile pen and extubated. Animals were observed for 7 days post injury and weighed on day 7.

Euthanasia: On day 7-post injury the animals were transcardially perfused and the brain fixed as follows. After sedation with ketamine, 20 mg/kg, i.m., the animal was

deeply anesthetized with 1-4% isoflurane. A sternal incision was made, the sternum cut with a bone cutter, and a rib spreader used to open the sternum and expose the heart. The right ventricle was catheterized and approximately one liter of blood was removed. The animals were then perfused with heparinized ice-cold saline, and Zamboni's fixative. When all cardiac activity ceased and perfusion was complete, the brain was removed and prepared for histology.

Results: One swine that was injured with the smaller gun recovered quickly, was extubated 7 minutes post injury, did not need analgesia and was returned to the pig run 42 minutes after injury. The second swine injured with the smaller gun had a slower recovery, was extubated 14 minutes post injury, was given 100 mg carprofen IM one hour post injury because of the slow recovery although did not display signs of pain and was returned to the pig run 3 hours post injury. The swine that was injured with the larger gun at 80 psi was extubated 12 minutes post injury, showed signs of pain by excessively grinding teeth and was given 100 mg carprofen one hour post injury. The swine were observed daily for 7 days for behavioral and coordination abnormalities. All observations were normal. Animals were weighed the day of injury and on day 7 prior to euthanasia. The two pigs that were injured with the smaller gun weighed 27 and 25 kg, respectively, on the day of injury and both weighed 32 kg on day 7 post injury. Their weight gains were 5 and 7 kg, respectively. The swine that was injured with the larger gun weighed 25 kg the day of injury and 26 kg on day 7-post injury, gaining only one kg.

Histology: Brains have been fixed. Histology has not been performed to date.

Cause-effect relationship: With the limited number of animals done to date, the animal injured with the larger gun showed signs of pain (grinding teeth), required analgesia for pain, and gained less weight in the 7 days post injury than the other group injured with the smaller gun which did not show signs of pain.

Problems: The large gun had a design deficiency that caused a failure. That has now been corrected.

KEY RESEARCH ACCOMPLISHMENTS:

- Protocol submitted to IACUC and approved on 3/20/2008.
- Protocol submitted to DOD March 2008.
- ACURO approval received on July 2009.
- Calibration of two different size air guns completed May 2008.
- Behavioral/learning/memory testing skills have been acquired.
- Three swine and one sham control swine underwent blast-induced traumatic brain injury using two different size air guns.
- Behavior monitored for one-week post blast-induced traumatic brain injury.
- Swine brains perfused *in situ* and prepared for histology.

REPORTABLE OUTCOMES:

An abstract and data from the air gun calibration experiments was submitted to the Military Health Research Forum September 2009.

CONCLUSION:

The air gun calibrations showed linear and reproducible results that quantify known air velocities through the use of air blast guns. Behavioral tests have been developed and will be successful in evaluating cognition in swine. Swine have received BI-TBI with air guns of two different sizes. The larger gun caused initial pain and reduced weight gain compared to the smaller gun. While this study is at an early stage, this swine model of blast induced traumatic brain injury will provide a needed tool to help in the early treatment of traumatic head injuries as well as possible improvements in military body armor.

REFERENCES:

None

APPENDICES:

None

SUPPORTING DATA:

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